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(54) Title: EUGENIA JAMBOLINA FRUIT EXTRACTS FOR TREATING DIABETES

(57) Abstract: A herbal therapeutic product for controlling diabetes comprising at least one hypoglycemic compound extracted from the pulp of fruit of Eugenia jambolina. The product is prepared by cleaning and drying the fruit, deseeding the fruit, soaking the de-seeded pulp in water, and keeping it overnight in cooled conditions, filtering the mixture, washing the residue with water to extract all the active compounds from the said mixture of water and pulp, reducing the water content by lyophilization to get the residues in concentrated form, purifying the said concentrated product via column chromatography. Thin layer chromatography is then used to get the hypoglycemic compounds in the form of bands. The bands are visualized using an iodine chamber. The hypoglycemic compounds are then extracted to get the herbal therapeutic product to control the glucose level.

EUGENIA JAMBOLINA FRUIT EXTRACTS FOR TREATING DIABETES

FIELD OF THE INVENTION

The present invention relates to an herbal therapeutic product for controlling diabetes mellitus comprising at least one hypoglycemic compound extracted from the pulp of a fruit from a species of genus Eugenia specifically Eugenia jambolina and a process for the preparation of the same.

The embodiment of the invention resides in the various compounds of the product and their mixture having immediate as well as longer lasting effects in controlling diabetes mellitus.

The other embodiment of the invention resides in four active hypoglycemic compounds comprising the product of the subject invention. These four active hypoglycemic compounds individually have the varying degrees of hypoglycemic characteristics, however, the combination thereof have been found to be most effective.

Still another embodiment of the invention resides in the discontinuity of such herbal therapeutic product for a predetermined period after controlling the diabetes of the patients as the hypoglycemic compounds of such products are having the property to control the glucose level, for longer durations.

It has been found that the intake of doses can be postponed for 2-3 days after controlling the glucose level because of longer lasting effects of these hypoglycemic compounds.

The source of carbohydrates in the diet has a significant influence on lipid(fat) metabolism in human being. An amount of sucrose is increased and the quantity of complex carbohydrates is decreased, the concentration of

cholesterol and more particularly of triglycerides increases which has a correlation between blood lipid concentration and impaired glucose tolerance.

One of the objects of the present invention is that the present invention is a 100% herbal product without any side effects.

Another object of the present invention is to treat the defect in metabolism, by increasing the activity of the glucose utilizing enzymes in liver, muscles and adipose tissues.

The present invention relates to the product obtained from the species of genus Eugenia having hypoglycemic effects in the blood sugar level.

A process for the preparation of the herbal therapeutic product to control the glucose level, comprises cleaning and drying the fruit of a species of family Eugenia to remove extraneous material from the outermost layer of the said fruit. De-seeding the fruit and soaking the said de-seeded fruit in water under controlled cooled conditions overnight to retain the activity of hypoglycemic compounds in the said mixture of water and pulp of de-seeded fruit. It is followed by mixing the said mixture to get the mixture of a uniform consistency.

DETAILED DESCRIPTION OF THE INVENTION:

Accordingly, the present invention relates to a herbal therapeutic product controlling diabetes mellitus comprising at least one hypoglycemic compound extracted from the pulp of a species of the genus Eugenia, specifically Eugenia jambolina.

The process for the preparation of such herbal product comprising cleaning and drying the fruit of a species of genus Eugenia of family Myrtaceae to remove the extraneous material from the outer layer of the said fruit,

deseeding the said fruit, soaking the de-seeded pulp in water, mixing the said mixture of water and pulp and keeping it overnight in a controlled cooled conditions to maintain the activity of hypoglycemic compounds, filtering the said mixture in the conventional manner, washing the residue with water to extract all the active compounds from the said mixture of water and pulp in the form of clear watery solution, reducing the water content by keeping the said clear watery solution in controlled freezing conditions by conventional means to get the residues in a concentrated form, purifying the said concentrated product in a chromatography by using slurries of different columns, adding buffers in the said purified mixture present in the said chromatography to elute the mixture of hypoglycemic compounds

The various steps involved in the aforementioned process are :

(a) The fruit of Eugenia jambolina is cleaned and dried to remove the extraneous material from the outer layer of the said fruit;

(b) The seed is removed from the said fruit in the conventional manner and is kept separately.

(c) The de-seeded pulp is soaked in (distilled) water and the same is mixed to get the uniform consistency of the liquid ;

(d) The said mixture is kept overnight in a controlled cooled conditions preferably between 4-10 degrees centigrade;

(e) The said mixture is filtered in the conventional manner

(f) The residue are washed with water to extract all the active compounds from the said pulp in the form of clear watery solution

(g) The water content are reduced by keeping the said clear watery solution in freezing conditions by conventional means to get the residues in concentrated form;

(h) The said concentrated product is purified by treating the same with slurries of solvents selected preferably from sephadex, diethyl amino ethyl cellulose, anion exchange resins or silica gel and distilled water in the chromatography;

(i) Buffers are added in the said purified mixture present in the chromatography to elute the hypoglycemic compounds from the said mixture.

The water content from said mixture can be reduced optionally to get the end product in solid form . The water is extracted from the said mixture after the stage of filtration by means of lypholizer.

The said pulp of Eugenia jambolina, is subjected to column chromatography for a period of 15 minutes -2 hours to obtain a hypoglycemic fraction at a slightly acidic pH of 5-7 preferably 6.0 to 6.5.

The said hypoglycemic fraction is subjected to thin layer chromatography using silica gel to form at least 2 visible bands . The R_f value of all the four bands is between 0.1-1.0, wherein the R_f value of one visible band which is having highly active hypoglycemic compound is between 0.5 to 0.7 and the R_f value of less active hypoglycemic compounds is between 0.1-0.2 and 0.9-1.0.

The pH of the column containing mixture of chemical compounds is maintained at pH of 6.0-8.0 preferably 6.5 to 7.5.

The said buffer used in the chromatography is selected preferably from the phosphate buffer, citrate buffer or TRIS buffer.

The pH of the said mixture of hypoglycemic compounds is to be maintained neutral.

The said mixture of hypoglycemic compounds were further analyzed to separate the active hypoglycemic compounds by means of thin layer chromatography to separate the different compounds in the form of separate bands. Such bands were further exposed to the iodine chamber for the clear demarcation of the individual compound. It has been found that out of 4 such hypoglycemic compounds present in the mixture, two compounds are having immediate effect in controlling and maintaining the blood sugar level , while other two hypoglycemic compounds have their effect after certain time.

In the thin layer chromatography, the said mixture of hypoglycemic compounds are dropped in the lower most end of a silica gel coated plate to get the deposition of said hypoglycemic compounds in the form of bands on the said silica gel coated plate by running with a mixture of solvents for 2-3 hours in the ascending manner. The said solvents are n-butanol, acetic acid and water in the ratio of 5:1:4. The silica gel coated plate with bands of hypoglycemic compounds is dried in cooled conditions and is exposed to iodine chamber to get the clear demarcation of the different hypoglycemic compounds bands which are extracted from the silica gel coated plate along with the fractions of silica gel. The extracted hypoglycemic compound along with silica gel is mixed with water for the separation of said silica gel by centrifugation.

The water used in the subject process is preferably distilled water.

A study was conducted to determine the effect of a herbal therapeutic product prepared from the pulp extracted from the genus Eugenia on the blood glucose, liver, aorta, pancreas and heart. The results were found very effective with no side effects which is the main embodiment of the present invention.

Various tests were conducted to analyze the effect of said product on testing the blood glucose level in fasting and in glucose tolerance test.

The test was conducted on alloxan induced diabetic rabbit where the said product prepared from the process of subject invention was given to alloxan induced diabetic rabbit after drawing the blood from fasting rabbits. After giving the dose of said product, the blood was withdrawn again from said rabbit after 90 minutes. After that 2 grams of glucose in a water solution was given to that rabbit. The blood was again withdrawn after two hours of glucose feed. It was found that said mixture reduced the blood glucose level even after the glucose feed, which was due to the rise in insulin level in the blood.

The said product was found to increase the activity of enzymes in liver, muscle and adipose tissue, which use glucose and thereby reduces blood glucose level. The said product was further found to reduce the level of lipids in blood which are responsible for heart diseases like total cholesterol, low density lipoprotein cholesterol, very low density lipoprotein cholesterol and triglycerides. At the same time it increases the level of favourable lipids for health such as high density lipoprotein cholesterol (HDLC). The said mixture of hypoglycemic compounds were found to be having no adverse effects on the liver and kidney functions.

No abnormalities were found after conducting the histopathology of liver, aorta, pancreas and heart, when the patient was treated with the product of subject invention.

Hence, the subject invention relates to a herbal therapeutic product to control the glucose level in diabetes mellitus and also minimizes the risk of heart diseases extracted from the pulp of a species from the genus Eugenia prepared from the process as herein before described, comprising said

hypoglycemic compound or a group of said hypoglycemic compounds in the form of a product.

EXAMPLES

EXAMPLE 1:

To prepare an herbal therapeutic product of present invention, 1 Kg of pulp of the fruit from the genus Eugenia is mixed in 500ml of distilled water and kept overnight in cooled conditions at 4°C , the said mixture is filtered by conventional manner, and the residue is washed twice or thrice to extract all the active compounds from the said mixture, the water from the resultant mixture is reduced by keeping the said resultant mixture in a lypholizer to get the residues in paste form, the chromatography of the said paste is done by washing the said paste with diethyl amino ethyl cellulose, a phosphate buffer is then added to elute the mixture of hypoglycemic compounds. The said hypoglycemic compounds were given in the form of solution mixed with water.

EXAMPLE 2:

An herbal therapeutic product of present invention is prepared by taking 500 grams of pulp of the fruit Eugenia Jambolina and mixing it in 200ml of distilled water which is kept overnight in cooled conditions at 4-10°C , the said mixture is filtered by conventional manner, and the residue is washed twice to extract all the active compounds from the said mixture, the water from the resultant mixture is extracted by keeping the said resultant mixture in a lypholizer to get the residues in paste form, the chromatography of the said paste is done by washing the said paste with a solvent sephadex, a phosphate buffer is then added to elute the mixture of hypoglycemic compounds, the said hypoglycemic compounds are dropped in very small quantity on the lower most end of a glass plate having silica coating on it, the said plate is placed in a glass chamber having solvents n-butanol, acetic acid and water in the ratio of 4:1:5, the bands of hypoglycemic compounds are deposited on the silica

coated plate by the capillary action, the plate with the deposition of hypoglycemic compounds is removed from the said glass chamber and is dried in cooled condition, then the plate is exposed to the iodine chamber to get the clear demarcation of the different hypoglycemic compounds bands which are extracted by scratching them off from the plate in the form of powder along with silica gel abstracts, the same is mixed with water for the separation of said silica gel by centrifugation.

We Claim:

1. An herbal therapeutic product for controlling diabetes mellitus comprising at least one hypoglycemic compound extracted from the pulp of species of Eugenia preferably Eugenia jambolina.

2 An herbal therapeutic product as claimed in claim 1, comprising four hypoglycemic compounds extracted from the pulp of species of Eugenia preferably Eugenia jambolina.

3. An herbal therapeutic product as claimed in claim 1, wherein the said pulp of species Eugenia, preferably Eugenia Jambolina, is subjected to column chromatography for a period of 15 minutes to 2 hours to obtain a hypoglycemic fraction at a slightly acidic pH of 5-7 preferably 6-6.5.

4. An herbal therapeutic product as claimed in claim 1, wherein the said hypoglycemic fraction is subjected to thin layer chromatography using silica gel to form at least 2 visible bands.

5. An herbal therapeutic product as claimed in claim 1, wherein the said hypoglycemic fraction obtained from thin layer chromatography is subjected to identification of different bands by exposing the said fraction to an iodine chamber.

6. An herbal therapeutic product as claimed in claim 4, wherein the said bands obtained have R_f value of between 0.1-1.0.

7. An herbal therapeutic product as claimed in claim 4, wherein the R_f value of highly active hypoglycemic compounds having immediate and longer lasting effects is between 0.5-0.7.

8. An herbal therapeutic product as claimed in claim 4, wherein the R_f value of less active hypoglycemic compounds is between 0.1-0.2.

9 An herbal therapeutic product as claimed in claim 4, wherein the R_f value of less active hypoglycemic compounds is between 0.9-1.0

10 A process for the preparation of an herbal therapeutic product extracted from the pulp of a species Eugenia jambolina , comprising, cleaning and drying the fruit to remove the extraneous material from the outer layer of the said fruit, deseeding the said fruit, soaking the de-seeded pulp in water, mixing the said mixture of water and pulp and keeping it overnight in controlled cooled conditions to maintain the activity of hypoglycemic compounds, filtering the said mixture by conventional manner, washing the residue with water to extract all the active compounds from the said mixture of water and pulp in the form of clear watery solution, reducing the water content by keeping the said clear watery solution in controlled freezing conditions by conventional means to get the residues in concentrated form, purifying the said concentrated product in a chromatography by using slurries of different columns, adding buffers in the said purified mixture present in the said chromatography to elute the mixture of hypoglycemic compounds, conducting thin layer chromatography of the said hypoglycemic compounds in the said mixture to get the said hypoglycemic compounds in the form of bands, exposing the said bands of the said hypoglycemic compounds in an iodine chamber for the clear demarcation of the said hypoglycemic compound bands and extracting such hypoglycemic compounds to get the herbal therapeutic product to control the glucose level.

11. A process for the preparation of an herbal therapeutic product as claimed in claim 10, where the said controlled cooled conditions for keeping overnight the mixture of water and pulp are from 4-10 degrees C.

12 A process for the preparation of an herbal therapeutic product as claimed in claim 10, wherein the water is extracted from the said mixture after the stage of filtration by means of lypholizer.

13 A process for the preparation of an herbal therapeutic product as claimed in claim 10, wherein the said slurries used in the chromatography are selected from sephadex, diethyl amino ethyl cellulose , anion exchange resins or silica gel.

14 A process for the preparation of an herbal therapeutic product as claimed in claim 10, wherein the said buffer used in the chromatography is selected from the phosphate buffer, citrate buffer or TRIS buffer.

15. A process for the preparation of an herbal therapeutic product as claimed in claim 10, where in the pH of the column containing mixture of chemical compounds is maintained at pH of 6.0-8.0 preferably 6.5 - 7.5.

16. A process for the preparation of an herbal therapeutic product as claimed in claim 10, where in the thin layer chromatography, the said mixture of hypoglycemic compounds are applied in the lower most end of a silica gel coated plate to get the deposition of said hypoglycemic compounds in the form of bands on the said silica gel coated plate by running with a mixture of solvents for 2-3 hours in the ascending manner.

17 A process for the preparation of an herbal therapeutic product as claimed in claim 10, wherein the said solvents are n-butanol, acetic acid and water in the ratio of 4:1:5 to 5:1:4.

18. A process for the preparation of an herbal therapeutic product as claimed in claim 16, wherein said silica gel coated plate with bands of hypoglycemic compounds is dried at cooled temperature.

19. A process for the preparation of an herbal therapeutic product as claimed in claim 18, wherein said dried plate is exposed to iodine in a closed chamber to get the clear demarcation of the different hypoglycemic compounds bands and extracting the same from said plate.

20 A process for the preparation of an herbal therapeutic product as claimed in claim 19, wherein said extracted hypoglycemic compound along with silica gel is mixed with water for the separation of said silica gel by centrifugation.

21 A process for the preparation of an herbal therapeutic product as claimed in claim 1, wherein the water used in any of the preceding claims is distilled water.

INTERNATIONAL SEARCH REPORT

International Application No
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K35/78 A61P5/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, EPO-Internal, PAJ, BIOSIS, EMBASE, MEDLINE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ACHREKAR S ET AL: "HYPOGLYCEMIC ACTIVITY OF EUGENIA-JAMBOLANA AND FICUS-BENGALENSIS: MECHANISM OF ACTION" IN VIVO (ATHENS), vol. 5, no. 2, 1991, pages 143-147, XP000608379 ISSN: 0258-851X abstract "Preparation of the plant extracts" on page 143 "Discussion" on page 146 --- -/--	1-21

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Pilling, S

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	KELKAR S M ET AL: "A SIMPLE TWO-STEP PURIFICATION OF ANTIDIABETIC COMPOUNDS FROM EUGENIA JAMBOLANA FRUIT-PULP: PROTEOLYTIC RESISTANCE AND OTHER PROPERTIES" PHYTOMEDICINE, vol. 3, no. 4, February 1997 (1997-02), pages 353-359, XP000940531 "Introduction" on page 353 "Purification of active compounds" on page 354 "Discussion" on page 358	1-21
X	KOHLI K R ET AL: "EUGENIA-JAMBOLANA: A PLANT DRUG WITH POTENTIAL ANTIDIABETIC PROPERTY (A REVIEW)" JOURNAL OF SCIENTIFIC RESEARCH IN PLANTS & MEDICINES, vol. 6, no. 1-4, 1985, pages 21-28, XP000940820 ISSN: 0253-7249 page 25, line 21 - line 22	1-21
X	US 5 886 029 A (DHALIWAL KIRPAL S) 23 March 1999 (1999-03-23) abstract column 2, line 59 - line 65 column 3, line 48 - line 50	1-21